Hindawi Publishing Corporation
Journal of Chemistry
Volume 2015, Article ID 894109, 10 pages
http://dx.doi.org/10.1155/2015/894109

Research Article

Low-Cost Biodegradation and Detoxification of Textile Azo Dye C.I. Reactive Blue 172 by *Providencia rettgeri* Strain HSL1

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Received 21 May 2015; Accepted 16 June 2015

Academic Editor: Mohd D. Rafatullah

Present study focuses on exploitation of agricultural waste wheat bran (WB) as growth medium for degradation of textile azo dye C.I. Reactive Blue 172 (RB 172) using a single bacterium *P. rettgeri* strain HSL1 (GenBank accession number JX853768.1). The bacterium was found to completely decolorize 50 mg L^{-1} of dye RB 172 within 20 h at 30 ± 0.2°C under microaerophilic incubation conditions. Additionally, significant reduction in COD (85%) and TOC (52%) contents of dye decolorized medium was observed which suggested its mineralization. Induction in the activities of azoreductase (159%) and NADH-DCIP reductase (88%) provided an evidence for reductive cleavage of dye RB 172. The HPLC, FTIR, and GC-MS analysis of decolorized products confirmed the degradation of dye into various metabolites. The proposed metabolic pathway for biodegradation of RB 172 has been elucidated which showed the formation of 2 intermediate metabolites, namely, 4-(ethenylsulfonyl) aniline and 1-amino-1-(4-aminophenyl) propan-2-one. The acute and phytotoxicity evaluation of degraded metabolites suggests that bacterial strain favors the detoxification of dye RB 172. Thus, WB could be utilized as a low-cost growth medium for the enrichment of bacteria and their further use for biodegradation of azo dyes and its derivatives containing wastes into nontoxic form.

1. Introduction

Synthetic textile dyes are of complex aromatic structures specially designed for chemical stability and versatility and to resist the effect of high temperature during wet processing operations which makes them highly recalcitrant [1]. Thousands of such synthetic dyes are extensively used in the textile industry for dyeing and printing purposes [2]. Approximately, 40–65 L of textile wastewater is produced per kg of cloth during dyeing processes [3]. Among all textile dyestuff used, the azo dyes constitute about 70% and are being used worldwide [4]. The discharge of azo dyes containing wastewaters into the environment may lead to the bioaccumulation which causes toxic effect on aquatic life and even carcinogenic and mutagenic effect on humans because of the conversion of azo group into aromatic amines [5, 6]. Aside from the human toxicity, colour of dyes interrupts the aquatic environment by reducing light penetration, gas solubility, and interference of phytoplankton's photosynthesis [7]. Therefore, treatment of textile wastewater becomes essential before discharging into the water streams. Additionally, limited supply and increasing cost of water for industrial sector have made the treatment and reuse of dyeing effluent mandatory to avoid the environmental pollution as well as reduce the production cost.

Several physicochemical methodologies such as coagulation and flocculation are most commonly used worldwide for treatment of textile effluent [8, 9]. But some shortcomings such as excessive use of chemicals, secondary pollution, large amount of sludge generation, low efficacies, and high operational cost discourage the employment of these methods [10]. Alternatively, the modern method bioremediation, which utilizes the ability of bacteria, fungi, or its combination system, has emerged as an effective method for the treatment of textile wastewaters [11–13]. However, higher price of microbial growth medium makes biological treatments expensive and beyond the use at commercial levels. Thus, to overcome the problem of higher cost of microbial growth medium and

make the bioremediation an efficient treatment technology, the use of agricultural waste as growth medium has been suggested.

A number of agricultural wastes and its by-products such as sugarcane bagasse, wheat straw, corn cob, rice bran, and wheat bran are cheapest and abundantly available carbon sources [14]. These are normally utilized as animal fodder and domestic fuel while a large portion is being disposed of as waste [15]. For instance, approximately 145.20 million tons per year of wheat straw is available in Asia [16]. However, only a small portion of wheat residues is used as animal feed and the rest is removed from the field by burning which causes air pollution and affects human health [17]. Recently, agricultural waste wheat bran has been used as growth medium for microbial consortium and their further use in biodegradation of azo dye Trypan Blue under submerged conditions [18].

In this view, the easily available agricultural waste wheat bran was further evaluated as a low-cost growth medium for degradation of model azo dye RB 172 using a single culture of *P. rettgeri* strain HSL1 bacterium under submerged conditions. Initially, the optimization of conditions for enhanced dye degradation efficacy was performed. The activities of dye degrading enzymes laccase, azoreductase, and NADH-DCIP reductase were assayed spectrophotometrically. Mineralization of dye was determined by the reduction in COD and TOC values whereas biodegradation was confirmed by HPLC, FTIR, and GC-MS analysis. Possible metabolic pathway for degradation of dye RB 172 has been constructed. Finally, the environmental risk assessment was performed by acute and phytotoxicity tests.

2. Materials and Methods

2.1. Chemicals and Textile Azo Dye RB 172. 2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), methyl red, nicotinamide adenine dinucleotide (NADH), and dichlorophenolindophenol (DCIP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Textile azo dye RB 172 (CAS number 85782-76-9; molecular formula = $C_{28}H_{26}N_6O_{10}S_3$; molecular weight = 702.74) was generously given by Mahesh Textile Processors (Ichalkaranji, MS, India).

2.2. Preparation of WB Medium. Agricultural waste wheat bran was obtained from local market (Kolhapur, MS, India), sieved, and oven-dried at 80°C ± 1.0°C for several hours until the weight was constant, and then 5 gm dry wheat bran was taken in 100 mL distilled water. This content was boiled for 15 min and the extract was separated by filtration through Whatman grade number 1 filter paper which then diluted to 100 mL with distilled water and called AWWB medium. The pH of AWWB medium was adjusted to 7.0, autoclaved for 15 min at 121°C, and used for further degradation experiments.

2.3. Preenrichment of P. rettgeri Strain HSL1. Prior to decolorization experiments the preenrichment of *P. rettgeri* strain HSL1 was routinely carried out in AWWB medium. A loopful of bacterial stock culture was inoculated in 250 mL Erlenmeyer flask containing 100 mL of AWWB medium (pH 7.0) and incubated at 30 ± 0.2°C for 24 h under shaking conditions (120 rpm). The overnight grown culture was then used as inoculum for further dye decolorization experiments.

2.4. Optimization of Decolorization Conditions. All the decolorization experiments were carried out in 250 mL Erlenmeyer flask containing 100 mL of preenriched *P. rettgeri* strain HSL1 culture. The optimization of conditions for enhanced decolorization of dye RB 172 was carried out by one parameter approach at a time. Initially, the effect of microaerophilic and aerobic incubation (shaking at 120 rpm), preenriched culture medium pH (3–12), incubation temperature (20, 30, 37, 40, and 50 ± 0.2°C), and dye concentrations (50–250 mg L^{-1}) was evaluated. At defined time of intervals the aliquots of culture supernatant (3 mL) were withdrawn and suspended particles were removed by adding equal volume of methanol followed by centrifugation (7500 ×g for 15 min, 4 ± 0.2°C) [13]. The resulted clear supernatant was analyzed for decolorization at maximum absorbance wavelength of 570 nm using UV-vis spectrophotometer (Hitahi U-2800; Hitachi, Tokyo, Japan). The control flasks which were without dye or bacterial culture were also tested under the same conditions. All the experiments were conducted at least in triplicate at 30 ± 0.2°C and average values were calculated. The decolorization was expressed in terms of percent using the formula:

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance}_{(0\,\text{h})} - \text{Observed absorbance after incubation}_{(t)}}{\text{Initial absorbance}_{(0\,\text{h})}} \times 100. \quad (1)$$

2.5. Dye Mineralization Analysis. The mineralization of dye RB 172 was confirmed by chemical oxygen demand (COD) and total organic carbon (TOC) analysis. For this, the control and decolorized culture broths were centrifuged (7500 ×g for 15 min, 4 ± 0.2°C) and filtered through 0.45 μm cellulose acetate filter (Sterlitech Corporation, Kent, WA, USA) to remove cell biomass. The reduction in COD was determined by dichromate closed reflux titrimetric method [19] and TOC by using a Sievers 5310C automated analyzer (GE Water & Process Technologies, Boulder, CO, USA).

2.6. Analysis of Metabolites after RB 172 Decolorization. The extraction of metabolites produced after degradation of RB 172 by *P. rettgeri* strain HSL1 was carried out by centrifugation (10,000 ×g for 20 min, 4 ± 0.2°C). The resulting supernatant was added into an equal volume of ethyl acetate and mixed vigorously to dissolve metabolites. The organic layer was separated, air-evaporated, and dried over anhydrous Na_2SO_4. The remaining metabolite residues were scrapped and dissolved in 3 mL of HPLC grade methanol. Finally, the sample was filtered through 0.45 μm cellulose acetate syringe filter (Sterlitech Corporation, Kent, WA, USA), evaporated to 250 μL in a fume hood, and subjected to HPLC, FTIR, and GC-MS analysis to confirm biodegradation.

HPLC analysis of control dye RB 172 and its decolorized metabolites were performed with Waters 2690 instrument (Waters Limited, Hertfordshire, UK) equipped with C_{18}

column (symmetry, 4.6 × 250 mm). The isocratic method using the methanol with a flow rate of 0.50 mL min^{-1} for 10 min and UV detector set at 280 nm was used [13]. A total of 10 μL of dye RB 172 dissolved in methanol and its degradation metabolites were manually injected into the column and elution profile was observed. FTIR analysis was done in the mid IR region of 600–4000 cm^{-1} with scan speed 16 using the Shimadzu 8400S spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The samples prepared with spectroscopic pure KBr were fixed in the sample holder and analyzed [13]. The identification of metabolites formed after decolorization was carried out using a QP2010 gas chromatography coupled with mass spectroscopy (Shimadzu Corporation, Kyoto, Japan). The ionization voltage was set at 70 eV and gas chromatography was performed in temperature programming mode with Restek column (0.25 mm × 30 m long). The initial column temperature was set at 40°C for 4 min, then increased linearly at 10°C/min to 270°C, and held for 4 min. Injection port temperature was 275°C and mass interface was maintained at 300°C. The helium with a flow rate of 1 mL/min was used as carrier gas for 30 min of run time [20].

2.7. Extraction and Activities of Biotransformation Enzymes. Extraction of enzymes after decolorization of dye RB 172 by *P. rettgeri* strain HSL1 and control medium (without dye) was carried out as per the procedure described earlier [21]. The bacterial cells were separated by centrifugation (7500 ×g for 15 min, 4 ± 0.2°C) and the resulted supernatant was considered test sample for determination of extracellular enzyme activities. The separated bacterial biomass was resuspended in 50 mM potassium phosphate buffer (pH 7.4), homogenized, and sonicated by giving 7 strokes of 30 s each for 2 min interval based on 50 amplitude output at 4 ± 1°C (Sonics-Vibracell ultrasonic processor). These sonicated cells were again centrifuged (7500 ×g for 15, 4 ± 0.2°C) and the supernatant was used as a source of intracellular enzymes. Similar protocol was followed to quantify the enzyme activities of control medium. Enzyme extracted from the culture medium without adding dye was considered control.

Activities of oxidoreductive enzymes such as laccase, azoreductase, and NADH-DCIP reductase were assayed spectrophotometrically at room temperature (30 ± 1°C). Laccase activity was determined by measuring the oxidation of ABTS at 420 nm (ε_{420} nm = 36000 (M cm)$^{-1}$) [22]. Determination of azoreductase activity was performed as per the procedure of Chen et al. [23], while NADH-DCIP reductase activity was assayed as reported previously [24]. All enzyme activity assays were conducted in triplicate and average rates were calculated. The protein content was determined by the method of Lowry et al. with bovine serum albumin as the standard [25].

2.8. Toxicity Studies. Environmental risk assessment of dye RB 172 and its degradation metabolites accumulation in animals was assessed by acute toxicity test with freshwater organism *Daphnia magna* as described elsewhere [11, 26]. The dye treated sample with *P. rettgeri* strain HSL1 was centrifuged (7500 ×g for 20 min, 4 ± 0.2°C), supernatant-collected, and sterilized by passing through 0.45 μm cellulose acetate syringe filter. The clear filtrate (100 mL) was taken into a 250 mL Erlenmeyer flask and five 24 h old neonates of *D. magna* were added. The tests were performed at 20 ± 0.2°C for 48 h in the absence of light and number of immobile organisms was counted after exposing to light for 20 seconds.

Toxicity of dye RB 172 and its degradation metabolites to plants was analyzed at room temperature on two kinds of economically important agricultural crops: *Sorghum vulgare* (monocot) and *Phaseolus mungo* (dicot) as described earlier [13]. Briefly, ten seeds of both plants were daily irrigated with 10 mL each of RB 172 (50 mg L^{-1}) and its degradation metabolites (50 mg L^{-1}). Length of shoot, root, and seed germination (%) was recorded after 13 days. Both the tests were conducted in triplicate with control in distilled water.

2.9. Statistical Analysis. One-way ANOVA was analyzed and Tukey-Kramer multiple comparison test was performed with GraphPad Prism to determine the significance of the parameter studied.

3. Results and Discussion

3.1. Decolorization of Textile Dye RB 172 in AWWB Medium. The preliminary investigation on WB as growth medium for decolorization of RB 172 by *P. rettgeri* strain HSL1 was carried out under microaerophilic conditions. The result of the UV-vis spectral analysis (400–800 nm) of the dye and its decolorized medium suggested that the *P. rettgeri* strain HSL1 treated medium (20 h) showed enhanced reduction in the absorbance indicating dye decolorization (Figure 1(a)). The removal of colour indicates that WB can be utilized as growth medium for decolorization of dyes which signifies the low-cost treatment approach. It is reported that *Providencia* sp. SRS82 could decolorize textile triazo dye Acid Black 210 in nutrient medium [20]. In addition, degradation of textile effluent by a developed bacterial consortium consisting of *Providencia* sp. SDS and *Pseudomonas aeruginosa* strain BCH has been reported in yeast extract medium [21]. As per our best knowledge, this is the first report showing the decolorization of textile azo dye by *Providencia* sp. using WB as growth medium under submerged conditions.

For the successful operation of biological wastewater treatment systems, the impact of aeration that provides oxygen for bacterial growth and stimulates its contact with medium substrates should be properly analyzed. Monitoring the efficiency under microaerophilic condition, *P. rettgeri* strain HSL1 showed >99% decolorization dye RB 172 (50 mg L^{-1}) within 20 h at 30 ± 0.2°C, whereas aerobic condition achieved only 12% performance within the same time and even 18% in 24 h (Figure 1(b)). These results indicate that aerobic condition strongly inhibited the decolorization of dye RB 172. Similar findings were reported in a previous study, where *Pseudomonas* sp. SUK1 exhibited higher decolorization rate of reactive azo dye Red BLI under microaerophilic condition whereas aerobic incubation showed only the growth but no decolorization [27]. It is reported that azoreductase

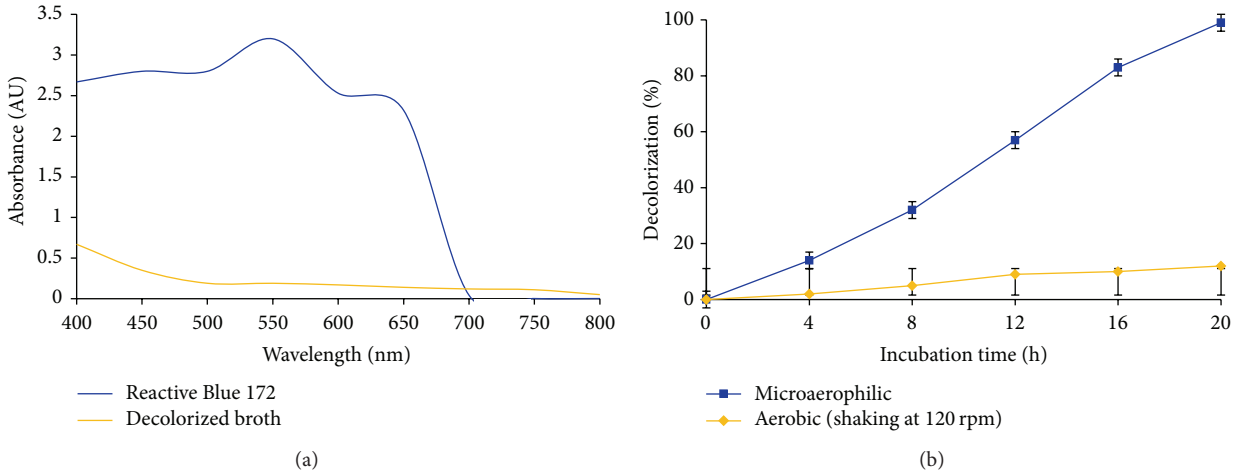


Figure 1: (a) UV-vis spectral analysis of control dye RB 172 and its decolorized broth by *P. rettgeri* strain HSL1. (b) Percentage of dye decolorization under microaerophilic and aerobic conditions. Data point represents the mean of three independent replicates; ±standard error of mean (SEM) is indicated by error bars.

is the key enzyme responsible for breakdown of azo bond of azo dyes and presence of oxygen normally inhibits the azo bond reduction [28]. Furthermore, aerobic condition may dominate the use of NADH and impedes the electron transfer from NADH to azo bonds resulting in the decreased decolorization performance [29]. Hence, in this study, further decolorization of azo dye RB 172 was carried out only in microaerophilic conditions.

3.2. Optimization of Decolorization Conditions. To scale up the decolorization process and provide an affordable treatment technology for textile wastewater, the optimization of decolorization conditions such as growth medium pH, incubation temperature, and dyes concentration was carried out. Result of the study demonstrated that bacterial strain could decolorize the dye at broad range of pH; however, the optimum pH was found to be 7.0 (Figure 2(a)). A significant decrease in the decolorization performance was observed at lower pH (3–5) and higher pH (9–12). The transport of dye molecules across cell membrane has been known to govern by pH of the medium, which is considered the rate limiting step in decolorization process [30].

The enhanced and maximum decolorization activity of dye RB 172 by bacterial culture was observed at 30 ± 0.2°C temperatures within 20 h of incubation in microaerophilic condition (Figure 2(b)). Further increase (37, 40, and 50°C) or decrease (20°C) in incubation temperature resulted in reduction in the decolorization performance. Effect of temperature on biodegradation of dyes might be associated with the microbial growth and enzymatic status of bacterial culture at respective conditions which determines its degradation abilities. Agrawal et al. reported that *Providencia* sp. SRS82 exhibited maximum dye decolorization activity for dye Acid Black 210 at 30°C temperature whereas lower and higher temperature than optimum have considerably decreased its decolorization rates [20].

The ultimate aim of wastewater treatment is to reduce the concentration of dyes. Result of the decolorization study at various concentrations (50–250 mg L^{-1}) showed that complete and rapid performance was observed at 50 mg L^{-1} within 20 h by *P. rettgeri* strain HSL1 (Figure 2(c)). The decolorization efficiency of bacterial culture was found to be decreased at dye concentration above 100 mg L^{-1}. It has been suggested that the concentration of dyes can influence the decolorization efficiency of bacteria due to the toxic effect imposed at higher concentrations [31].

3.3. Dye Mineralization Analysis. The efficacy of textile wastewater treatment is determined by the mineralization of dye molecules in terms of decrease in COD and TOC contents [32]. Result of the dye decolorization by *P. rettgeri* strain HSL1 at optimum conditions, that is, WB medium pH 7.0, incubation temperature 30 ± 0.2°C, 50 mg L^{-1} of dye concentration, and microaerophilic incubation, suggests that the complete decolorization with significant reduction in COD (85%) and TOC (52%) was observed within 20 h (Table 1). These decreased magnitudes of analyzed parameter suggest the applicability of WB medium for growth of *P. rettgeri* strain HSL1 and their use in mineralization of azo dye RB 172. Additionally, the remained agricultural residues after preparation of WB medium could be used as low-cost adsorbent for dye removal and subsequent degradation by SSF [33]. But the SSF based methods work better with water soluble dyes as dye must adsorb on solid substrate prior to degradation. This signifies the importance of our work over several studies where biodegradation of textile dye was carried out using nutrient medium [34, 35]. It is well known that cost of growth medium used has strong influence on overall bioremediation economics. The market price of wheat bran displayed on the world's biggest online commerce company http://www.alibaba.com/ is US $154–162/metric ton, while the cost of mostly used defined growth

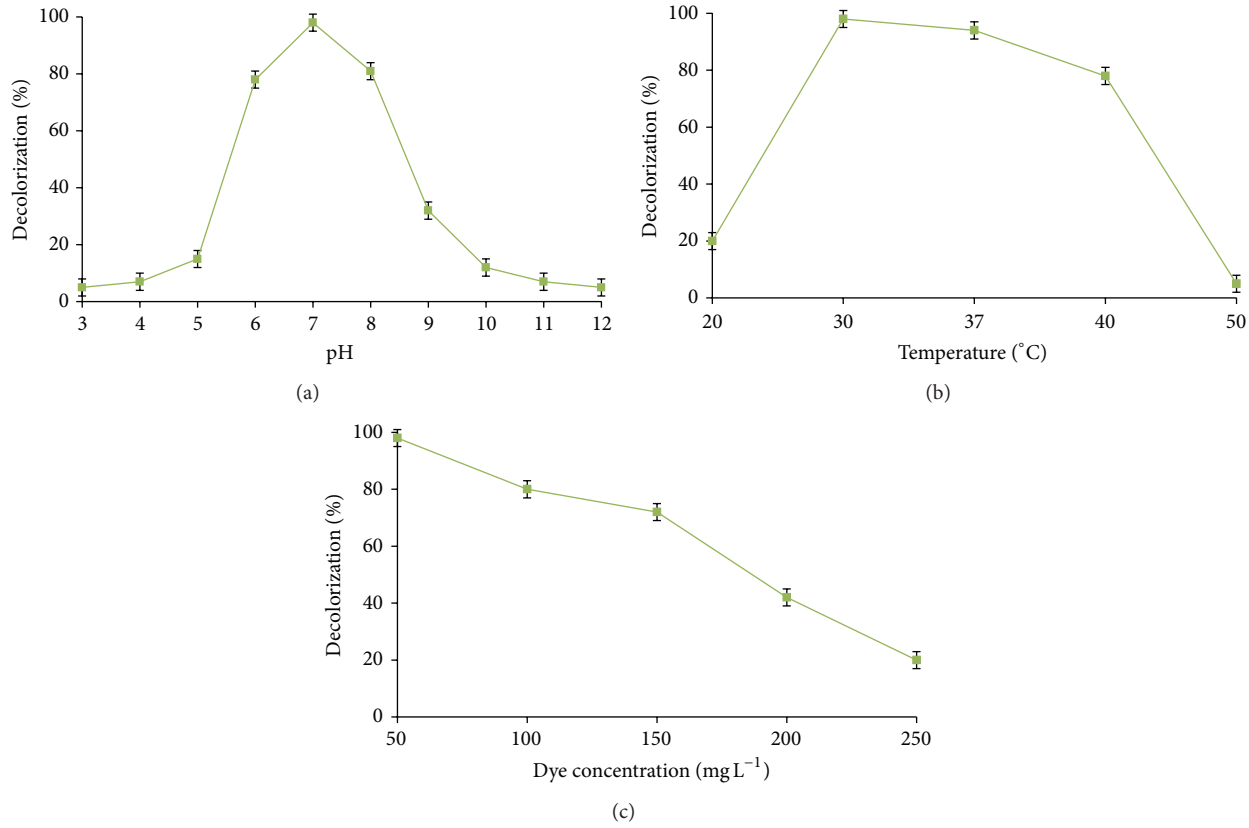


Figure 2: (a) Effect of culture medium pH, (b) incubation temperature, and (c) initial dye concentrations on the percentage of dye RB 172 decolorization by *P. rettgeri* strain HSL1. Data point represents the mean of three independent replicates; ±SEM is indicated by error bars.

Table 1: Analysis of control dye RB 172 and its decolorized broth after treatment with *P. rettgeri* strain HSL1.

Parameters	Control dye	Treated (after 20 h)
COD (mg L^{-1})	1020 ± 5.0	153 ± 3.0
TOC (mg L^{-1})	1587 ± 7.0	762 ± 4.0
Colour removal (%)	0	99 ± 1.0

Values are mean of three experiments ± standard deviation (SD).

Table 2: Enzyme activities during decolorization of dye RB 172 by *P. rettgeri* strain HSL1.

Enzymes	Control cells (0 h)	After decolorization (20 h)
Laccase[1]	0.285 ± 0.04	0.456 ± 0.05*
Azo reductase[2]	0.162 ± 0.02	0.420 ± 0.03*
NADH-DCIP reductase[3]	17 ± 2.05	32 ± 2.12*

Values are mean of three experiments ± standard error of mean (SEM), significantly different from control cells at $^{*}P < 0.001$ by one-way analysis of variance (ANOVA) with Tukey-Kramer comparison test.
[1]μM of ABTS oxidized min^{-1} mL of $enzyme^{-1}$ mg of $protein^{-1}$.
[2]μM of methyl red reduced min^{-1} mL of $enzyme^{-1}$ mg of $protein^{-1}$.
[3]μM of DCIP reduced min^{-1} mL of $enzyme^{-1}$ mg of $protein^{-1}$.

medium nutrient broth is US $5000–20000/metric ton. This huge difference in price of wheat bran and nutrient medium signifies the importance of our work for designing affordable biological wastewater treatment processes.

3.4. Enzyme Analysis. Results of the enzyme activity analysis suggest that *P. rettgeri* strain HSL1 possesses laccase, azo reductase, and NADH-DCIP reductase enzyme system in control cells. On the other hand, significant induction in the activities of laccase (60%), azo reductase (159%), and NADH-DCIP reductase (88%) from decolorized medium cells indicates its active involvement in breakdown of dye RB 172 (Table 2). Higher induction in the activity of azoreductase as compared to laccase highlights the dominance of reductive enzymes in decolorization process. Lade et al. reported the involvement of azo reductase in enzymatic cleavage of azo dye Trypan Blue by bacterial consortium [18]. Additionally, the roles of oxidoreductive enzymes in the decolorization of reactive azo dye Red HE3B have also been characterized in *Providencia* sp. SDS [21].

3.5. Biodegradation Analysis. The HPLC analysis of control dye showed the presence of one major peak at retention time of 2.702 min and three minor peaks at retention times of 2.125, 2.801, and 3.394 min (Figure 3(a)). After the dye decolorization process, the disappearance of peaks as seen in case of the control and the formation of completely different three major peaks at retention times of 2.521, 3.241, and

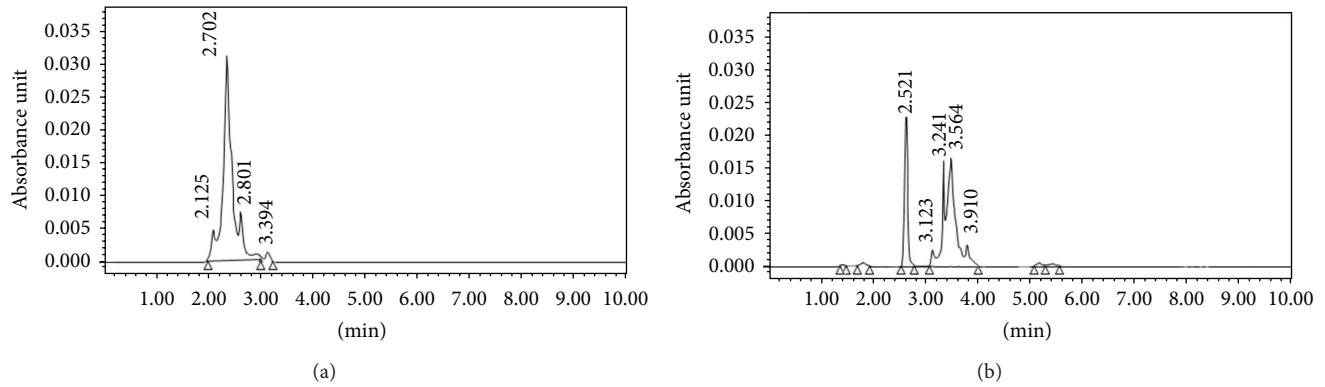


FIGURE 3: (a) HPLC chromatogram of the control dye RB 172 and (b) its decolorized products obtained after treatment with *P. rettgeri* strain HSL1.

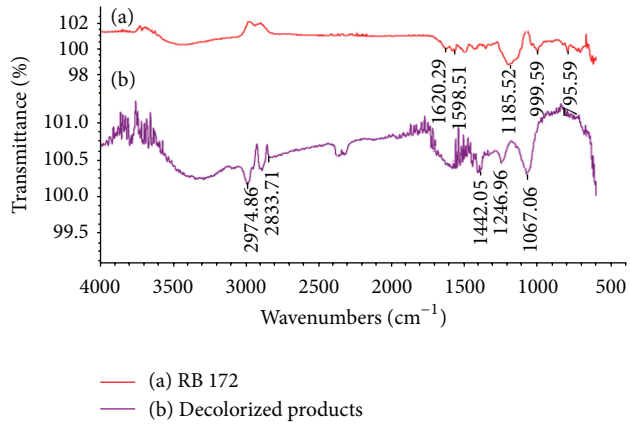


FIGURE 4: FTIR spectrum of (a) control dye RB 172 and (b) its decolorized products obtained after treatment with *P. rettgeri* strain HSL1.

3.564 min and two minor peaks at retention time of 3.123 and 3.910 min were observed (Figure 3(b)). The appearance of new minor peaks and disappearance of the major peak in the decolorized dye products elution profile support the biodegradation of RB 172.

The FTIR spectrum of control dye RB 172 compared with extracted products is shown in Figure 4. The FTIR spectrum of the control dye exhibits specific peaks at 1620.29 and 1598.51 cm^{-1} due to the presence of azo groups –N=N– stretching (Figure 4(a)). The peak at 1185.52 cm^{-1} corresponds to S=O stretching of sulfonyl chlorides while the presence of peak at 999.59 cm^{-1} showed P–O stretching as in phosphorus compounds.

The FTIR spectrum of extracted products after decolorization of dye RB 172 showed variation in the positions of peaks when compared to control dye spectrum. The disappearance of peaks at 1620.29 and 1598.51 cm^{-1} indicates the reductive cleavage of dye RB 172 at azo bond position (Figure 4(b)). The peak at 2974.86 cm^{-1} indicates the C–H stretching of alkanes while the peak at 2833.71 cm^{-1} shows C–H stretching of ethers. The peak obtained at 1442.05 cm^{-1} is due to C–H deformation of alkanes. In addition, peak at 1246.96 cm^{-1} shows O–NO_2 vibration of nitrates while the peak at 1067.06 cm^{-1} suggests C–OH stretching of primary alcohols. These changes in the FTIR spectrum are clear evidence for the degradation of dye RB 172 into simpler molecules, like aliphatic amines and carboxylic acids. Additionally, significant induction in the activities of azoreductase and laccase suggested initial reductive cleavage of azo bond of dye RB 172 and further breakdown of formed metabolites.

The GC-MS analysis was carried out to identify the metabolites formed during decolorization of RB 172 by bacterial strain. The gas chromatogram of degraded dye metabolites showed the presence of several peaks; however, only two peaks were identified by mass spectrum at retention times of 19.54 and 23.10 min (Figure 6). The structure of identified compounds assigned from fragmentation pattern and m/z values obtained indicates the formation of 4-(ethenylsulfonyl) aniline and 1-amino-1-(4-aminophenyl) propan-2-one as low molecular weight degradation metabolites.

The pathway for RB 172 biodegradation by *P. rettgeri* strain HSL1 has been proposed showing the possible metabolites produced (Figure 5). The GC-MS analysis and enzyme activities suggested the initial reductive cleavage of azo bond which yields 4-(ethenylsulfonyl) aniline (m/z 183) via formation of 3,4-diamino-6-{[4-(1-amino-2-oxo-propyl)-phenylazo]-methyl}-5-hydroxy-naphthalene-2,7-disulfonic acid (MW 523.54) as unidentified metabolite. The significant induction in the activity of azoreductase and disappearance of azo peak in the FTIR spectrum of decolorized products also support the reduction of dye RB 172. It is known that azoreductase is responsible for the reductive cleavage of azo bond which results in dye decolorization [36]. The unidentified metabolite [I] is supposed to be further cleaved at azo position to form low molecular weight compound 1-amino-1-(4-aminophenyl) propan-2-one (m/z 165) as final product via azoreductase activity.

3.6. Toxicity Analysis. The treated textile wastewaters are being commonly discharged into the environmental sinks. Hence, it becomes essential to assess the risk of treated

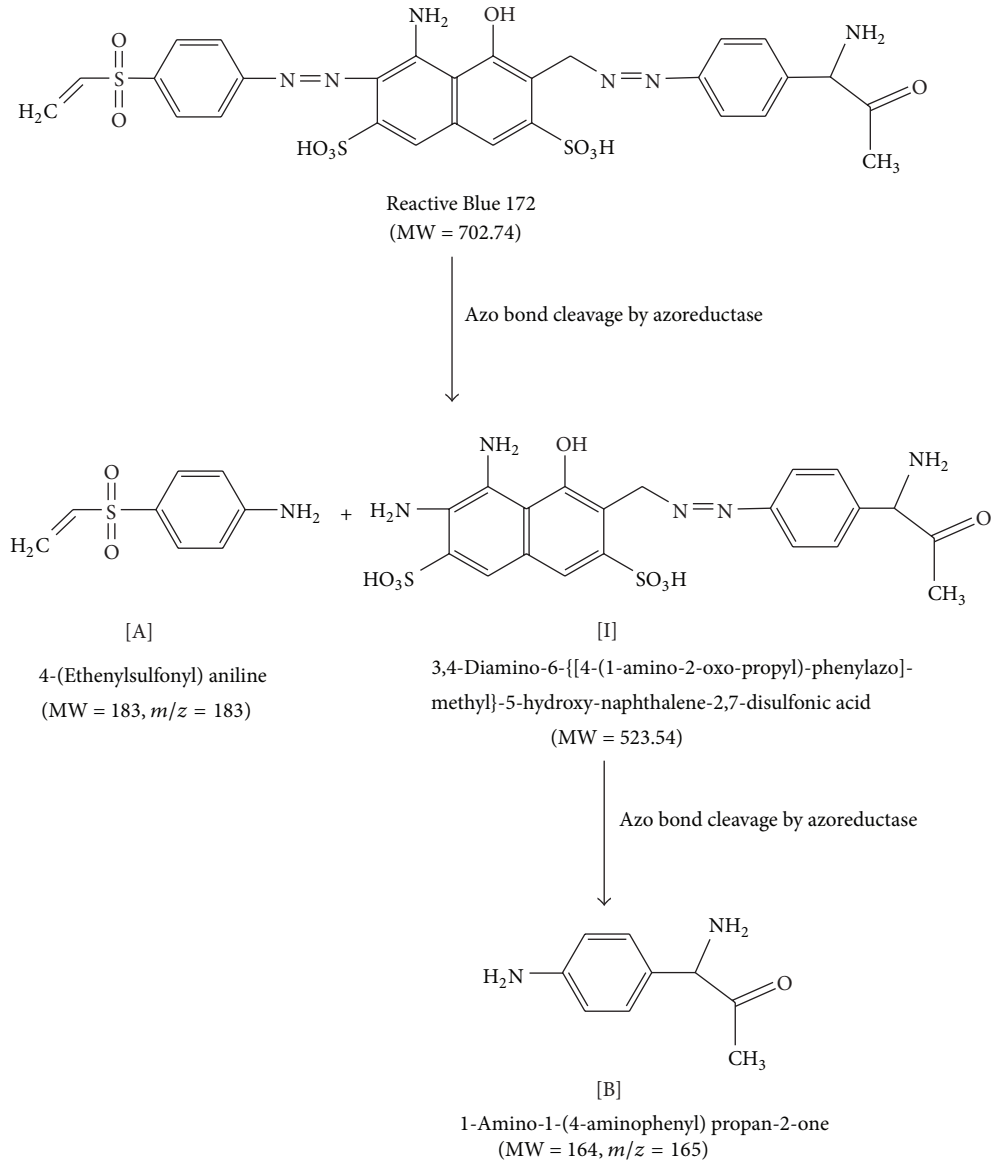


Figure 5: Proposed metabolic pathway for the biodegradation of dye RB 172 by *P. rettgeri* strain HSL1.

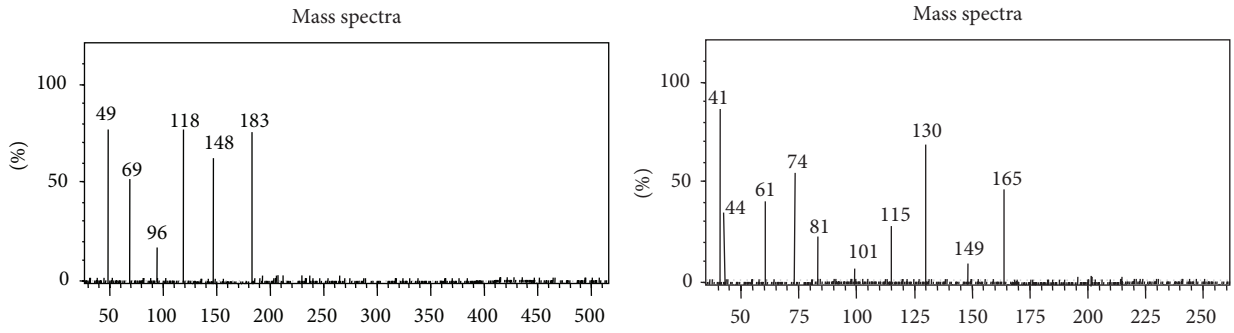
wastewaters for animal and plants with high accuracy and ecological relevance. The acute and phytotoxicity assays are advocated as essential tools for addressing these issues [26, 35]. Acute tests with *D. magna* have been suggested as a primary screening method for the evaluation of lethal toxicity of chemicals to mammals and humans [37]. Result of the acute test showed 100% mortality of *D. magna* in untreated dye RB 172 (50 mg L^{-1}) solution suggesting the toxic nature of dye (Table 3). The acute toxicity is assumed to occur in test organisms when the accumulated dye content equals a critical concentration. In contrast, the treatment of dye RB 172 with *P. rettgeri* strain HSL1 was sufficient to completely detoxify the dye as no mortality of *D. magna* was observed in treated samples.

Result of the phytotoxicity analysis revealed inhibition of germination for each seed of *S. vulgare* and *P. mungo* by 70 and 60%, respectively, treated with 50 mg L^{-1} of dye RB 172 solution (Table 4). However, near about 90% germination was observed in both the seeds irrigated with dye degradation metabolites. Additionally, good elongation of shoot (9.2 and 10.2 cm) and root (3.6 and 4.1 cm) lengths for *S. vulgare* and *P. mungo,* respectively, was observed in dye

Table 3: Mortality of *D. magna* exposed to dye RB 172 and its culture supernatants obtained after degradation by *P. rettgeri* strain HSL1.

Samples	Mortality (%)
Distilled water	0 ± 0.0
RB 172 (50 mg L^{-1})	45 ± 2.0
Treated dye medium	0 ± 0.0

Values are mean of three experiments ± SD.



m/z: 183
Retention time (min): 19.54
Metabolites: 4-(Ethenylsulfonyl) aniline [I]

m/z: 165
Retention time (min): 23.10
Metabolites: 1-Amino-1-(4-aminophenyl) propan-2-one [II]

Figure 6: GC-MS analysis of metabolites obtained after decolorization of dye RB 172 by *P. rettgeri* strain HSL1.

Table 4: Phytotoxicity of the dye RB 172 and its metabolites obtained after degradation by *P. rettgeri* strain HSL1.

Samples	*S. vulgare*			*P. mungo*		
	Germination (%)	Shoot length (cm)	Root length (cm)	Germination (%)	Shoot length (cm)	Root length (cm)
Distilled water	100	9.5 ± 0.5	3.8 ± 0.3	100	10.4 ± 0.4	4.5 ± 0.2
RB 172 (50 mg L^{-1})	30	4.5 ± 0.2*	2.2 ± 0.1*	40	5.8 ± 0.2*	2.1 ± 0.3*
Degradation metabolites	90	9.2 ± 0.4	3.6 ± 0.4	90	10.2 ± 0.3	4.1 ± 0.2

Values are mean of three experiments ± SE.
Seeds germinated in dye are significantly different from control (distilled water) at $^{*}P < 0.001$ by one-way analysis of variance (ANOVA) with Tukey-Kramer comparison test.

degradation metabolites grown plants. The strong influence of physiological characteristics in untreated dye grown plants suggests that dye RB 172 has toxic effect on plants as it inhibited germination and affected shoot and root elongation. The overall findings of the degradation study and toxicity analysis demonstrated that *P. rettgeri* strain HSL1 is not only able to decolorize the dye RB 172 but also completely detoxify it. This suggests the future application of *P. rettgeri* strain HSL1 for low-cost biodegradation as well as detoxification of azo dye contaminated wastewaters.

4. Conclusions

Wheat bran was successfully utilized as the growth medium for degradation of dye RB 172 by using *P. rettgeri* strain HSL1. A real market cost analysis of WB with defined growth medium nutrient broth suggests that WB could be used as a low-cost growth medium for bioremediation processes. The low-cost wheat bran medium, rapid degradation, and complete detoxification of model azo dye by *P. rettgeri* strain HSL1 revealed an economical and ecofriendly approach for designing azo dye containing wastewater treatment technologies. However, further studies are required to explore the use of WB medium for growth of bacteria and their use in the treatment of real textile effluent at reactor scale, which is an objective of our future research.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Harshad Lade performed the actual work and wrote the paper. Sanjay Govindwar and Diby Paul supervised the work.

Acknowledgment

The authors would like to thank all the anonymous referees for their constructive comments and suggestions.

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